

## Race 4 identification of *Fusarium oxysporum* f. sp. *cubense* from Cavendish cultivars in Hainan province, China

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**Abstract.** *Fusarium oxysporum* f. sp. *cubense* from Hainan province of South China was identified by colony morphology on modified Komada medium (K2 medium). Forty isolates of *F. oxysporum* f. sp. *cubense* obtained from Cavendish cultivars sampled at random from 20 banana farms of Hainan province, China, formed lacinated colonies on modified K2 medium. None of the isolates of *F. oxysporum* f. sp. *cubense* sampled from 10 Gros Michel cultivars in five other banana farms in Hainan with the same method and six other species of *Fusarium* conserved in our laboratory formed lacinated colonies on the same medium. The results showed that it is a simple, fast and reliable method for identifying *F. oxysporum* f. sp. *cubense* Race 4.

Panama disease in banana, caused by *Fusarium oxysporum* f. sp. *cubense*, is one of the diseases that has caused the greatest damage to banana crops worldwide throughout the 20th century (Stover 1972). The highly susceptible cultivar, Gros Michel, used by growers until 1960, was replaced by clones of the Cavendish Subgroup. Although Cavendish cultivars are resistant to Race 1 and Race 2 of the pathogen, Race 4 damages these cultivars in subtropical banana-growing regions (Su *et al.* 1977). During surveys conducted in 2005 and 2006, a high incidence of Banana fusarium wilt on Cavendish cultivars was observed in Hainan province of South China. A rapid pathogenicity test using small seeding Gros Michel had previously been developed (Stover 1959), which was useful for rapid identification of *F. oxysporum* f. sp. *cubense*, but not for races of this fungus. We used colony morphology on modified K2 medium (Sun *et al.* 1978) to identify Race 4 of *F. oxysporum* f. sp. *cubense* in Hainan, China.

Forty isolates of *F. oxysporum* f. sp. *cubense* obtained from Cavendish cultivars sampled at random from 20 banana farms of Hainan province, China, and 10 isolates obtained from Gros Michel cultivars sampled from five other banana farms in Hainan with the same method, and six other species of *Fusarium* conserved in our laboratory, were tested on modified K2 medium.

The modified K2 medium consists of a basal medium and 100 mL of solution. The basal medium contains the following compounds in 900 mL of distilled water: K<sub>2</sub>HPO<sub>4</sub>, 1 g; KCl, 0.5 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g; FeNaEDTA, 0.01 g; L-asparagine 2 g; galactose 10 g, and 16 g of agar. After autoclaving, the basal medium was mixed with 100 mL of solution containing the following agents: PCNB (pentachloronitrobenzene, 75% WP),

0.9 g; oxgall, 0.5 g; Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·0.10H<sub>2</sub>O 0.5 g; streptomycin sulfate 0.3 g. The medium was adjusted to pH 3.8 ± 0.2 with 10% phosphoric acid. Six plates per treatment were used and colonies were observed after 15-day incubation at 28°C under fluorescent light. The experiment was repeated three times.

All 40 isolates of *F. oxysporum* f. sp. *cubense* obtained from Cavendish cultivars formed lacinated colonies on modified K2 medium that were distinct from those of isolates from 10 Gros Michel cultivars and six other *Fusarium* spp. (Fig. 1). The number of rays produced per colony ranged from 20 to 50 and the colonies appeared yellowish when observed from the bottom (Fig. 2). None of the isolates from Gros Michel cultivars and from six other *Fusarium* spp. formed lacinated radial colonies on the same medium. All the isolates of *F. oxysporum* f. sp. *cubense* from Cavendish cultivars, and 10 isolates from Gros Michel cultivars and six other *Fusarium* spp. did not produce lacinated colonies on potato-dextrose agar.

The Cavendish cultivars are susceptible only to race 4 (Su *et al.* 1977). According to the colony morphology on modified K2 medium (Sun *et al.* 1978), 40 isolates of *F. oxysporum* f. sp. *cubense* obtained from Cavendish cultivars from 20 banana farms of Hainan province, China, were race 4. These results further support the reliability of the method for identification of *F. oxysporum* f. sp. *cubense* race 4, at least for the isolates from Hainan. This may reflect the short history of the fungus in Hainan. It is likely that the fungus originated from a single introduction from Taiwan. However, it is possible that a clone of race 4 without lacinated colonies may be found in the future.



**Fig. 1.** Colony morphology of *F. oxysporum* f. sp. *cubense* race 4 on modified K2 medium, showing colonies of isolates from Cavendish cultivars (upper) and isolates from isolates from Gros Michel cultivars and other *Fusarium* spp. (lower).



**Fig. 2.** Colony morphology of *F. oxysporum* f. sp. *cubense* race 4 on modified K2 medium, showing that the colonies of isolates from Cavendish cultivars appeared yellowish when observed from the bottom. Those colonies of laciniated appearance are *F. oxysporum* f. sp. *cubense* race 4.

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